a1 cont October 17, 2000, the contents of all of which are hereby incorporated by reference, in their entirety, into the present application.--.

Please replace the paragraph at page 4, lines lines 14-16, beginning "Figure 3:", with the following rewritten paragraph:

--Figure 3: Shows the nucleotide (SEQ ID NO.: 1) and complete amino acid sequence (SEQ ID NO.: 2) encoding human CTLA4 receptor fused to the oncostatin M signal a2 peptide, and including the newly identified N-linked glycosylation site, as described in Example 1, infra.--.

Please replace the paragraph at page 6, lines 20-25, please replace the paragraph beginning "Figure 7:", with the following rewritten paragraph:

--Figure 17: Depicts sequencing alignment of CD28 and CTLA4 family members. Sequences of human (H) (SEQ ID NO.: 7), mouse (M) (SEQ ID NO.: 5), rat (R) (SEQ ID a3 NO.: 6), and chicken (Ch) CD28 (SEQ ID NO.: 8) are aligned with human (SEQ ID NO.: 3) and mouse CTLA4 (SEQ ID NO.: 4). The signal peptides are underlined with a dashed line. The transmembrane domains are underlined with a solid line. The CDR-analogous regions are noted. The dark shaded areas highlight complete conservation of residues while the light shaded areas highlight conservative amino acid substitutions in all family members.--.

Please replace the paragraph at page 7, lines 12-13, beginning "Figure 22:", with the following rewritten paragraph:

--Figure 22: Depicts the nucleotide (SEQ ID NO.: 9) and amino acid sequence of a a4 CTLA4Ig (SEQ ID NO.: 10) having wildtype extracellular domain of CTLA4.--.

Please replace the paragraph at page 7, lines 15-17, beginning "Figure 23:", with the following rewritten paragraph:

--Figure 23: Depicts the nucleotide (SEQ ID NO.: 11) and amino acid (SEQ ID NO.: 12) a5 sequences of L104EIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position –1 to aspartic acid at position +124.--.

Please replace the paragraph at page 7, lines 19-21, beginning "Figure 24:", with the following rewritten paragraph:

--Figure 24: Depicts the nucleotide (SEQ ID NO.: 13) and amino acid sequence (SEQ ID NO.: 14) of L104EA29YIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position –1 to aspartic acid at position +124.--.

a6

Please replace the paragraph at page 7, lines 23-25, beginning "Figure 25:", with the following rewritten paragraph:

--Figure 25: Depicts the nucleotide (SEQ ID NO.: 15) and amino acid sequences (SEQ ID NO.: 16) of L104EA29LIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position –1 to aspartic acid at position +124.--.

a7

Please replace the paragraph at page 7, lines 27-29, beginning "Figure 26:", with the following rewritten paragraph:

--Figure 26: Depicts the nucleotide (SEQ ID NO.: 17) and amino acid sequences (SEQ ID NO.: 18) of L104EA29TIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position –1 to aspartic acid at position +124.--.

a8

Please replace the paragraph at page 8, lines 1-3, beginning "Figure 27:", with the following rewritten paragraph:

--Figure 27: Depicts the nucleotide (SEQ ID NO.: 19) and amino acid sequences (SEQ ID NO.: 20) of L104EA29WIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position –1 to aspartic acid at position +124.--.

a9

Please replace the paragraph at page 9, lines 22-25, beginning "Figure 37:", with the following rewritten paragraph:

--Figure 37: Depicts the results of a FACS assay, showing L104EIg and L104ES25RIg bind CHO cells stably transfected with human CD80 or CD86. A) L104EIg and

a10

a10 cont L104ES25RIg bind to human CD80 CHO-transfected cells; B) L104EIg and L104ES25RIg bind to human CD86 CHO-transfected cells--.

Please replace the paragraph at page 11 lines 1-8, beginning "One embodiment", with the following rewritten paragraph:

--One embodiment of a soluble CTLA4 has been deposited with the American Type Culture Collection (ATCC) in Manassas, Maryland, under the provisions of the Budapest Treaty on May 31, 1991 and has been accorded ATCC accession number: 68629. ATCC 68629 is DNA encoding ***CTLA4***Ig. Additionally, the CTLA4Ig-24 CHO cell line has been deposited with the ATCC under the Budapest Treaty on May 31, 1991 and has been accorded accession number ATCC 10762. DNA encoding L104EA29YIg was submitted for deposit on June 19, 2000 with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209. The DNA encoding L104EA29YIg has been accorded ATCC accession number PTA-2104.--

a11

Please replace the paragraph at page 33, lines 2-21, beginning "Because a signal peptide", with the following rewritten paragraph:

--Because a signal peptide for CTLA4 was not identified in the CTLA4 gene, the N-terminus of the predicted sequence of CTLA4 was fused to the signal peptide of oncostatin M (Malik et al., Mol. and Cell. Biol. 9:2847 (1989)) in two steps using overlapping oligonucleotides. For the first step, the oligonucleotide, CTCAGTCTGGTCCTTGCACTCCTG

a12 TTTCCAAGCATGGCGAGCATGGCAATGCACGTGGCCCAGCC (SEQ ID NO.: 21) (which encoded the C terminal 15 amino acids from the oncostatin M signal peptide fused to the N terminal 7 amino acids of CTLA4) was used as forward primer, and TTTGGGCTCCTGATCAGAATCTGGGCACGGTTG (SEQ ID NO.: 22) (encoding amino acid residues 119-125 of the amino acid sequence encoding CTLA4 receptor and containing a Bcl I restriction enzyme site) as reverse primer. The template for this step was cDNA synthesized from 1 micro g of total RNA from H38 cells (an HTLV II infected T cell leukemic cell line provided by Drs. Salahudin and Gallo, NCI, Bethesda, MD). A portion of

the PCR product from the first step was reamplified, using an overlapping forward primer, encoding the N terminal portion of the oncostatin M signal peptide and containing a Hind III restriction endonuclease site, CTAGCCACTGAAGCTTCACCAATGGGTGTACTGCTCACACAGAGGACGCTGCT CAGTCTGGTCCTTGCACTC (SEQ ID NO.: 23) and the same reverse primer. The product of the PCR reaction was digested with Hind III and Bcl I and ligated together with a Bcl 1/Xba I cleaved cDNA fragment encoding the amino acid sequences corresponding to the hinge, CH2 and CH3 regions of IgCγ1 into the Hind III/Xba I cleaved expression vector, CDM8 or Hind III/Xba I cleaved expression vector pLN--.

a12 cont

Please replace the paragraph at page 33, lines 23-29, beginning "A schematic map", with the following rewritten paragraph:

--A schematic map of the resulting CTLA4Ig fusion construct is shown in Figure 1. Sequences displayed in this figure show the junctions between CTLA4 (upper case letters, unshaded regions) and the signal peptide, SP, of oncostatin M (dark shaded regions), and the hinge, H, of IgC(gamma)1 (stippled regions). The amino acid in parentheses was introduced during construction. Asterisks (*) indicate cysteine to serine mutations introduced in the IgCγ hinge region. The immunoglobulin superfamily V-like domain present in CTLA4 is indicated, as are the CH2 and CH3 domains of IgC(gamma)1.--

a13

Please replace the paragraph at page 34, lines 21-27, beginning "CTLA4Ig", with the following rewritten paragraph:

--CTLA4Ig was purified by protein A chromatography from serum-free conditioned supernatants (Figure 2). Concentrations of CTLA4Ig were determined assuming an extinction coefficient at 280 nm of 1.6 (experimentally determined by amino acid analysis of a solution of known absorbance). Molecular weight standards (lanes 1 and 3, Figure 2) and samples (1 micro grams) of CTLA4Ig (lanes 2 and 4) were subjected to SDS-PAGE (4-12% acrylamide gradient) under non-reducing conditions (- beta ME, lanes 1 and 2) or

a14

a14 cont reducing conditions (+ beta ME, lanes 3 and 4) Proteins were visualized by staining with Coomassie Brilliant Blue. --.

Please replace the paragraph at page 35, lines 19-31, beginning "Because of expression of CTLA4 receptor", with the following rewritten paragraph:

--Because the expression of CTLA4 receptor protein in human lymphoid cells has not been previously reported, it was necessary to locate a source of CTLA4 mRNA. cDNA was reverse transcribed from the total cellular RNA of H38 cells, as noted above, was used for cloning by PCR. For this purpose, the oligonucleotide, GCAATGCACGTGGCCCAGCCTGCTGTGGTAGTG (SEQ ID NO.: 24) (encoding the first 11 amino acids in the predicted coding sequence) was used as a forward primer, and TGATGTAACATGTCTAGATCAATTGATGGGAATAAAATAAGGCTG (SEQ ID NO.: 25) (homologous to the last 8 amino acids in CTLA4 and containing a Xba I site) as reverse primer. The template again was a cDNA synthesized from 1 micro gram RNA from H38 cells. Products of the PCR reaction were cleaved with the restriction endonucleases Nco I and Xba I and the resulting 316 bp product was gel purified. A 340 bp Hind III/Nco I fragment from the CTLAIg fusion described above was also gel-purified, and both restriction fragments were ligated into Hind III/Xba I cleaved CDM8 to form OMCTLA.--.

a15

Please replace the paragraph at page 36, lines 21-25, beginning "Receptor-immunoglobulin C gamma", with the following rewritten paragraph:

--Receptor-immunoglobulin C gamma (IgCγ) fusion proteins B7Ig and CD28Ig were prepared as described by Linsley et al., in J. Exp. Med. 173:721-730 (1991), incorporated by reference herein. Briefly, DNA encoding amino acid sequences corresponding to the respective receptor protein (e.g. B7) was joined to DNA encoding amino acid sequences corresponding to the hinge, CH2 and CH3 regions of human IgCγ1. This was accomplished as follows.--.

a16

Please replace the paragraph at page 37, lines 6-25, beginning "Plasmid Construction.", with the following rewritten paragraph:

--Plasmid Construction. Expression plasmids containing cDNA encoding CD28, (as described by Aruffo and Seed, Proc. Natl. Acad. Sci. USA 84:8573 (1987)), were provided by Drs. Aruffo and Seed (Mass General Hospital, Boston, MA). Plasmids containing cDNA encoding CD5, (as described by Aruffo, Cell 61:1303 (1990)), were provided by Dr. Aruffo. Plasmids containing cDNA encoding B7, (as described by Freeman et al., J. Immunol. 143:2714 (1989)), were provided by Dr. Freeman (Dana Farber Cancer Institute, Boston, MA). For initial attempts at expression of soluble forms of CD28 and B7, constructs were made (OMCD28 and OMB7) as described by Linsley et al., J. Exp. Med., supra, in which stop codons were introduced upstream of the transmembrane domains and the native signal peptides were replaced with the signal peptide from oncostatin M (Malik et al., Mol. Cell Biol. 9:2847 (1989)). These were made using synthetic oligonucleotides for reconstruction (OMCD28) or as primers (OMB7) for PCR. OMCD28, is a CD28 cDNA modified for more efficient expression by replacing the signal peptide with the analogous region from oncostatin M. CD28Ig and B7Ig fusion constructs were made in two parts. The 5' portions were made using OMCD28 and OMB7 as templates and the oligonucleotide, CTAGCCACTGAAGCTTCACCATGGGTGTACTGCTCACAC (SEQ ID NO.: 26) (encoding the amino acid sequence corresponding to the oncostatin M signal peptide) as a forward primer, and either TGGCATGGGCTCCTGATCAGGCTTAGAAGGTCCGGGAAA (SEQ ID NO.: 27) or, TTTGGGCTCCTGATCAGGAAAATGCTCTTGCTTGGTTGT (SEQ ID NO.: 28) as reverse primers, respectively. Products of the PCR reactions were cleaved with restriction endonucleases (Hind III and BclI) as sites introduced in the PCR primers and gel purified--

a17

Please replace the paragraph at page 37, line 27, through page 38, line 9, beginning "The 3' portion of the fusion constructs", with the following rewritten paragraph:

--The 3' portion of the fusion constructs corresponding to human IgCγ1 sequences was made by a coupled reverse transcriptase (from Avian myeloblastosis virus; Life Sciences

a18

Associates, Bayport, NY)-PCR reaction using RNA from a myeloma cell line producing human-mouse chimeric mAb L6 (provided by Dr. P. Fell and M. Gayle, Bristol-Myers Squibb Company, Pharmaceutical Research Institute, Seattle, WA) as template. The oligonucleotide, AAGCAAGAGCATTTTCCTGATCAGGAGCCCAAATCTTCTGACAAAACTCACA CATCCCCACCGTCCCCAGCACCTGAACTCCTG (SEQ ID NO.: 29) was used as forward primer, and CTTCGACCAGTCTAGAAGCATCCTCGTGCGACCGCGAGAGC (SEQ ID NO.: 30) as reverse primer. Reaction products were cleaved with BclI and XbaI and gel purified. Final constructs were assembled by ligating HindIII/BclI cleaved fragments containing CD28 or B7 sequences together with BclI/XbaI cleaved fragment containing IgCγ1 sequences into HindIII/XbaI cleaved CDM8. Ligation products were transformed into MC1061/p3 E. coli cells and colonies were screened for the appropriate plasmids. Sequences of the resulting constructs were confirmed by DNA sequencing. --.

a18 cont

Please replace the paragraph at page 38, lines 17-24, beginning "CD5Ig was constructed in identical fashion, using", with the following rewritten paragraph:

-- CD5Ig was constructed in identical fashion, using CATTGCACAGTCAAGCTTCCATGCCCATGGGTTCTCTGGCCACCTTG (SEQ ID NO.: 31) as forward primer and ATCCACAGTGCAGTGATCATTTGGATCCTGGCATGTGAC (SEQ ID NO.: 32) as reverse primer. The PCR product was restriction endonuclease digested and ligated with the IgCγ 1 fragment as described above. The resulting construct (CD5Ig) encoded a mature protein having an amino acid sequence containing amino acid residues from position 1 to position 347 of the sequence corresponding to CD5, two amino acids introduced by the construction procedure (amino acids DQ), followed by DNA encoding amino acids corresponding to the IgCγ 1 hinge region. --.

a19

Please replace the paragraph at page 39, lines 21-30, beginning "Immunostaining and FACS^R Analysis.", with the following rewritten paragraph:

--Immunostaining and FACS^R Analysis. Transfected CHO or COS cells or activated T cells were analyzed by indirect immunostaining. Before staining, CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with murine mAbs 9.3 (Hansen et al., Immunogenetics 10:247 (1980)) or BB-1 (Yokochi et al., J. Immunol. 128:823 (1981)), or with Ig fusion proteins (all at 10 micro grams/ml in DMEM containing 10% FCS) for 1-2 h at 4 °C. Cells were then washed, and incubated for an additional 0.5-2h at 4 °C with a FITC-conjugated second step reagent (goat anti-mouse Ig serum for murine mAbs, or goat anti-human Ig Cγ serum for fusion proteins (Tago, Inc., Burlingame, CA)). Fluorescence was analyzed on a FACS IV^R cell sorter (Becton Dickinson and CO., Mountain View, CA) equipped with a four decade logarithmic amplifier.--

a20

Please replace the paragraph at page 40, line 28, through page 41, line 5, beginning "mAbs.", with the following rewritten paragraph:

--mAbs. Murine monoclonal antibodies (mAbs) 9.3 (anti-CD28) and G19-4 (anti-CD3), G3-7 (anti-CD7), BB-1 (anti-B7 antigen) and rat mAb 187.1 (anti-mouse kappa chain) have been described previously (Ledbetter et al., Proc. Natl. Acad. Sci. 84:1384-1388 (1987); Ledbetter et al., Blood 75:1531 (1990); Yokochi et al., supra) and were purified from ascites before use. The hybridoma producing mAb OKT8 was obtained from the ATCC, Rockville, MD, and the mAb was also purified from ascites before use. mAb 4G9 (anti-CD19) was provided by Dr. E. Engleman, Stanford University, Palo Alto, CA). Purified human-mouse chimeric mAb L6 (having human Cγ1 Fc portion) was a gift of Dr. P. Fell and M. Gayle (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA).--

a21

Please replace the paragraph at page 41, lines 7-15, beginning "Immunostaining and FACS^R Analysis.", with the following rewritten paragraph:

--Immunostaining and FACS^R Analysis. Prior to staining, COS or CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with mAbs or Ig fusion proteins at 10 micro grams/ml in DMEM containing 10% FBS for 1-2 hr at 4 degrees C. Cells were then washed, and incubated for an additional 0.5-2 hrs at 4 degrees C with FITC-conjugated goat anti-mouse immunoglobulin or with FITC-conjugated goat anti-human IgCγ serum (both from Tago, Burlingame, CA). When binding of both mAbs and Ig fusion proteins were measured in the same experiment, FITC-conjugated anti-mouse and anti-human second step reagents were mixed together before use. Fluorescence on a total of 10,000 cells was then analyzed by FACS^R.--

a22

Please replace the paragraph at page 44, lines 10-17, beginning "Binding of CTLA4Ig on B7 Positive CHO cells. ", with the following rewritten paragraph:

--Binding of CTLA4Ig on B7 Positive CHO cells. To further characterize the binding of CTLA4Ig and B7, the binding activity of purified CTLA4Ig on B7$^+$ CHO cells and on a lymphoblastoid cell line (PM LCL) was measured in the experiment shown in Figure 5. Amplified transfected CHO cell lines and PM LCLs were incubated with medium only (no addition) or an equivalent concentration of human IgCγ1-containing proteins (10 micro grams/ml) of CD5Ig, CD28Ig or CTLA4Ig. Binding was detected by FACS^R following addition of FITC-conjugated goat anti-human Ig second step reagents. A total of 10,000 stained cells were analyzed by FACS^R.--

a23

Please replace the paragraph at page 46, lines 14-19, beginning "Primary mixed lymphocyte reaction (MLR)", with the following rewritten paragraph:

--Primary mixed lymphocyte reaction (MLR) blasts were stimulated with irradiated T51 lymphoblastoid cells (LC) in the absence or presence of concentrations of murine mAb 9.3 Fab fragments, or B7Ig, CD28Ig or CTLA4Ig immunoglobulin Cγ fusion proteins. Cellular

a24

a24 Cont

proliferation was measured by [^{3}H]-thymidine incorporation after 4 days and is expressed as the percentage of incorporation by untreated cultures (21,000 cpm). Figure 9 shows the mean of quadruplicate determinations (SEM $\leq$ 10%).--.

Please replace the paragraph at page 48, lines 8-17, beginning "These results demonstrate", with the following rewritten paragraph:

--These results demonstrate the first expression of a functional protein product of CTLA4 transcripts. CTLA4Ig, a fusion construct containing the extracellular domain of CTLA4 fused to an IgCγ1 domain, forms a disulfide-linked dimer of M_r approximately 50,000 subunits. Because no interchain disulfides would be predicted to form in the Ig portion of this fusion, it seems likely that cysteines from CTLA4 are involved in disulfide bond formation. The analogous CD28Ig fusion protein (Linsley et al, supra, 1991) also contains interchain disulfide linkage(s). These results suggest that CTLA4 receptor, like CD28 (Hansen et al., Immunogenetics 10:247-260 (1980)), exists on the T cell surface as a disulfide linked homodimer. Although CD28 and CTLA4 are highly homologous proteins, they are immunologically distinct, because the anti-CD28 mAb, mAb 9.3, does not recognize CTLA4 (Figures 4 and 5).--

a26

Please replace the paragraph at page 57, lines 20-23, beginning "In addition, two mutants", with the following rewritten paragraph:

--In addition, two mutants encoding the residues P103A and Y104A (MYPPAY (SEQ ID NO.: 40) and MYPPPA (SEQ ID NO.: 41), respectively) from the CD28Ig 99MYPPPY104 hexapeptide using CD28Ig as a template were also prepared by the same method.--.

a27

Please replace the paragraph at page 57, line 30, through page 58, line 1, beginning "These primers encoded the following sequences:", with the following rewritten paragraph:

--These primers encoded the following sequences:

a28

CDM8FP:5'-AATACGACTCACTATAGG (SEQ ID NO.: 33)

a28 Cont

CDM8RP:5'-CACCACACTGTATTAACC (SEQ ID NO.: 34)

Please replace the paragraph at page 58, line 29, through page 59, line 2, beginning "HS7, HS8, and HS9 constructs", with the following rewritten paragraph:

--HS7, HS8, and HS9 constructs were prepared by replacing a ~350 base-pair HindIII/HpaI 5' fragment of HS4, HS4-A, and HS4-B, respectively, with the equivalent cDNA fragment similarly digested from HS5 thus introducing the CDR1-like loop of CTLA4 into those hybrids already containing the CTLA4 CDR3-like region.--.

a29

Please replace the paragraph at page 62, lines 29-31, beginning "Several versions of the model", with the following rewritten paragraph:

--Several versions of the model with modified assignments of some residues to β-strands or loops were tested using 3D-profile analysis (Luthy et al., 1992, Nature 336:83-85) in order to improve the initial alignment of the CTLA4 extracellular region sequence with an IGSF variable fold.--

a30

Please replace the paragraph at page 63, lines 12-15, beginning "Regions of sequence conservation", with the following rewritten paragraph:

--Regions of sequence conservation are scattered throughout the extracellular domains of these proteins with the most rigorous conservation seen in the hexapeptide MYPPPY (SEQ ID NO.: 35) motif located in the **CDR**3-like loop of both CTLA4 and CD28 (Figure 17). This suggests a probable role for this region in the interaction with a B7 antigen, e.g., B7-1 and B7-2.--.

a31

Please replace the TABLE B at page 73, lines 1-45, beginning "**TABLE B.** Binding of CTLA4 and CD28 monoclonal antibodies", with the following rewritten paragraph:

--**TABLE B.** Binding of CTLA4 and CD28 monoclonal antibodies to CTLA4Ig and CD28Ig mutant fusion proteins and to CTLA4/CD28Ig hybrid fusion proteins.

a32

	anti-CTLA4 mAbs			anti-CD28 mAb
	7F8	11D4	10A8	9.3
CTLA4Ig MUTANT FUSION PROTEIN				
AYPPPY (SEQ ID NO.: 36)	+++	+++	+++	-
MAPPPY (SEQ ID NO.: 37)	++	+	++	-
MYAPPY (SEQ ID NO.: 38)	+	-	+	-
MYPAPY (SEQ ID NO.: 39)	+++	++++++	+++	-
MYPPAY (SEQ ID NO.: 40)	+++	-	+	-
MYPPPA (SEQ ID NO.: 41)	+++	++	+++	-
AAPPPY (SEQ ID NO.: 42)	+	++	+++	-
CD28Ig MUTANT FUSION PROTEIN				
MYPPAY (SEQ ID NO.: 40)	-	-	-	-
MYPPPA (SEQ ID NO.: 41)	-	-	-	+
CTLA4/CD28Ig HYBRID FUSION PROTEINS				
HS1	-	-	-	-
HS2	-	-	-	+
HS3	-	-	-	-
HS4	-	-	-	+++
HS5	-	-	-	-
HS6	+	-	-	-
HS4-A	-	-	-	++
HS4-B	-	-	-	++
HS7	-	-	-	+++
HS8	-	+	-	+++
HS9	-	+	-	-
HS10	-	-	-	-
HS11	-	-	-	+
HS12	-	-	-	-
HS13	-	-	-	-
HS14	-	-	-	-
CTLA4Ig	+++	+++	+++	-
CD28Ig	-	-	-	+++

Antibody binding was rated from that seen for wild type protein (+++) to above background (+), and no detectable binding (-).

Please replace the paragraph at page 77, lines 12-17, with the following rewritten paragraph:

--Experimental data were first fit to a model for a single ligand binding to a single receptor (1-site model, i.e., a simple langmuir system, A+B<->AB), and equilibrium association constants (K_d=[A]•[B]\[AB]) were calculated from the equation $R=R_{max}•C/(K_d+C)$. Subsequently, data were fit to the simplest two-site model of ligand binding (i.e., to a receptor having two non-interacting independent binding sites as described by the equation $R=R_{max1}•C\(K_{d1}+C)+R_{max2}•C\(K_{d2}+C)$.--

a33

Please replace the paragraph at page 84, lines 23-28, beginning "From tyrosine +23 to threonine +30,", with the following rewritten paragraph:

--From tyrosine +23 to threonine +30, a pool of degenerate forward primers were generated having the following sequence:

a34

5' CGA GGC ATC GCT AGC TTT GTG TGT GAG XXK XXK XXK XXK XXK XXK XXK XXK GAG GTC CGG GTG ACA GT 3' (SEQ ID NO.: 43)

Where X = any of the four nucleotides A, T, G, or C and K = either T, G, or C

Each primer contained a Nhe I restriction enzyme cut site.--

Please replace the paragraph at page 84, lines 30-32, beginning "The reverse primer had the following sequence:", with the following rewritten paragraph:

--The reverse primer had the following sequence:

a35

5' GGT TGC CGC ACA GAC TTC GGT CAC CTG GCT GTC AGC CTG CCG AAG CAC TGT CAC CCG GA 3' (SEQ ID NO.: 44)--

Please replace the paragraph at page 86, lines 24-31, beginning "Five mutants were enriched through these 5 rounds of panning.", with the following rewritten paragraph:

--Five mutants were enriched through these 5 rounds of panning.

a36

Mut 9 F-E-P-K-R-G-V-Q (SEQ ID NO.: 45)

Mut 19 W-D-Q-Y-T-G-Y-G (SEQ ID NO.: 46)

Mut 71 W-D-A-Y-R-N-Q-Q (SEQ ID NO.: 47)